REMARKS

Favorable reconsideration is respectfully requested in view of the foregoing amendments and the following remarks.

I. EXAMINER INTERVIEW, CLAIM STATUS & AMENDMENTS

Claims 8, 9 and 11-16 were pending in this application when last examined, and stand rejected.

Applicant thanks Examiners Salmon and Meyers for the recent telephonic discussions and interview.

Claims 8, 9 and 11-16 are pending and rejected.

Claim 8 has been amended to replace "no quenching" language with "reduced quenching" as suggested in the interview. As acknowledged by the Examiners in the interview and at the bottom of page 6 of the Office Action, the Specification provides working examples demonstrating and supporting "reduced quenching" when a probe of the present invention interacts and hybridizes with a target. For instance, see the description of the results in Table 1 on pages 13-14 of disclosure, which shows increased fluorescent intensity as a result of "reduced quenching" for the probes of the claimed invention.

No new matter has been added.

II. CLAIM OBJECTION

In item 4 on page 2 of the Office Action, claim 8 was newly objected to on the basis that "absorbs" in line 8 of claim 8 should be changed to "absorb."

This objection is respectfully traversed. Kindly note that "absorbs" is the correct tense of the verb as it relates to the noun "energy-absorbing substance", which is singular, not plural. Therefore, the objection is untenable and should be withdrawn.

III. INDEFINITENESS REJECTION

In item 5 on page 3 of the Office Action, claims 8-9 and 11-16 were newly rejected under 35 U.S.C. § 112, second paragraph, as indefinite on the basis that the claim preamble of "detecting a nucleic acid" does not correspond to the last step of "measuring energy released from the labeling substance."

The present amendment overcomes this rejection. In particular, claim 8 has been amended to include in the last step of the claim the recitation "wherein the released energy indicates detection of the target nucleic acid" to correlate the measuring step with "detecting a nucleic acid" in the preamble.

IV. ENABLEMENT REJECTION

In item 6 on pages 8-9 of the Action, claims 8-9 and 11-16 were rejected under 35 U.S.C. § 112, first paragraph, on the basis that the specification lacks enablement for the claimed invention wherein "no quenching" is indicative of the claimed invention. It appears that the Office does not believe the invention will work to show increased fluorescence due to no quenching when the target nucleic acid is detected/hybridized to the probe. The Office bases the rejection on the fact that the art appears to teach the opposite where reduced fluorescence occurs due to quenching when the probe hybridizes to the target.

This rejection is respectfully traversed as applied to the amended claims.

The test of enablement is whether one reasonably skilled in the art can make or use the invention based on the disclosure in the specification coupled with the knowledge in the art without undue experimentation. See M.P.E.P. § 2164.01.

As noted during the interview, it appears that the Office has misunderstood how the present invention works with respect to the quenching mechanism.

Firstly, "quenching" means a decrease in fluorescence that results from an absorption of some or all of the emission energy, but does not mean the disappearance of fluorescence emitted

from a fluorescent material. Specifically, it is self-evident that the energy absorbing substance cannot absorb all the energy released from the labeling substance. In other words, fluorescence would not completely disappear even when the energy absorbing substance exists close to the labeling substance. Please see the attached relevant pages of the Dictionary of Biochemistry and Molecular Biology, which support Applicants' position.

Secondly, the phrase "no quenching" is intended to mean interruption of quenching, but does not mean complete fluorescence or complete "no quenching." This interpretation can be justified by the quenching mechanism. Specifically, the hybridization of the probe with the target nucleic acid occurs under an equilibrium condition and therefore a very small amount of the probe does not hybridize with the target nucleic acid. Therefore, the reduced fluorescence intensity can be observed even when the probe hybridizes with the target nucleic acid.

Thus, in the present invention, the energy-absorbing substance in the probes specifically interacts with the double-stranded nucleic acid due to the hybridization of the probe with a target nucleic acid, thereby resulting in reduced quenching of the labeling substance. In other words, normally, the labeling substance releases energy and the energy absorbing substance absorbs this energy from the labeling substance. However, when the nucleic acid hybridizes with the target DNA, the energy transfer between the labeling substance and the energy-absorbing substance stops (*i.e.*, reduced quenching occurs). Thus, it is respectfully submitted that the disclosure fully supports the "no quenching" language.

In the interview, the Examiners acknowledged these arguments and suggested amending the claims to replace "no quenching" with "reduced quenching" as such language is supported by the disclosure and accurately reflects the present invention.

Without intending to acquiesce to the rejection and for the sole purpose of expediting prosecution, claim 8 has been amended to replace "no quenching" language with "reduced quenching" as suggested in the interview. As acknowledged by the Examiners in the interview and at the bottom of page 6 of the Office Action, the Specification provides working examples

demonstrating and supporting "reduced quenching" when a probe of the present invention interacts and hybridizes with a target.

For instance, please see the description of the results in Table 1 on pages 13-14 of disclosure, which shows increased fluorescent intensity as a result of "reduced quenching" for the probes of the claimed invention. For example, Nos. 14, 17, 21 and 24 in Table I correspond to cases where both labeling substance and the energy-absorbing substance are linked to probes and where homologous sequences are provided in the solutions. As demonstrated on page 14, lines 15-22, the probes of the present invention as shown in Nos. 14, 17, 21 and 24 show an increase in fluorescent intensity upon hybridization. In other words, in samples using probes into which pyrene was introduced, fluorescent intensity increased only when a double-stranded chain was formed between probe and target (Nos. 14, 17, 21, and 24, among Nos. 13-24).

Thus, it is respectfully submitted that the disclosure fully supports the amended language of "reduced quenching."

During the interview, the Examiners newly raised the issue of what is meant by a probe having the labeling substance positioned on the nucleic acid 0 to 1 nucleotides apart from the energy-absorbing substance.

In reply thereto, it is respectfully submits that there is support for the claimed probe having the labeling substance on the nucleic acid at a position 0 to 1 nucleotides apart from the energy-absorbing substance. Please see the arguments on pages 8-9 of the response filed May 10, 2006 for a discussion about this language. As noted in this prior response, Example 1 (at page 12, lines 20-30) is an example of a probe wherein the energy-absorbing substance (pyrene) is 0 nucleotides apart from the labeling substance. See the probe designated EFN1-FP at line 27 on page 12 of the Specification. See also line 28 at page 12, probe EFN2-FP, which is a probe where the absorbing substance is 1 nucleotide apart from the labeling substance.

In further reply thereto, please see the discussion on pages 3-4 of the response filed June 20, 2006, which discuss Probe Nos. 14, 17, 21 and 24 in Table 1 on page 13 of the disclosure.

Again, Nos. 1 to 12 in Table 1 on page 13 correspond to a probe having only the labeling substance, while Nos. 13 to 24 in Table 1 correspond to a probe having the labeling substance and the energy-absorbing substance.

Nos. 1 to 6 and 13 to 18 correspond to a probe targeted for the polynucleotide EC1, while Nos. 7 to 12 and 19 and 24 correspond to a probe targeted for the polynucleotide EC2.

Nos. 1 to 3, 7 to 9, 13 to 15, and 19 to 21 correspond to a probe, wherein the labeling substance is located 0 nucleotides apart from the energy-absorbing substance. Nos. 4 to 6, 10 to 12, 16 to 18, and 22 to 24 correspond to a probe, wherein the labeling substance is located 1 nucleotide apart from the energy absorbing substance.

Nos. 14, 17, 21, and 24 correspond to cases where both the labeling substance and the energy-absorbing substance are linked to probes and where their homologous sequences are provided in the solutions.

The chemical structures of the modified parts in Nos. 14 and 17 are shown in Figure 3.

Based on such disclosure, it is respectfully submitted that the skilled artisan would clearly understand what is meant by a probe having the labeling substance positioned on the nucleic acid 0 to 1 nucleotides apart from the energy-absorbing substance.

Regarding item 6 of the Office Action, it was indicated that it is unclear how the present method differs from the method taught in the prior art. Applicants respectfully submit that the closest prior art is Livak et al. (PCR Methods and Applications, Vol. 4, pp. 357-362, 1995), which is cited in the Office Action issued on December 21, 2005. However, the present invention is completely different from the method disclosed in Livak et al.

Specifically, the method in Livak et al. utilizes the TaqMan™ method, *i.e.*, the 5' nuclease PCR assay. More specifically, the method in Livak et al. employs a 5' nuclease reaction by a Taq DNA polymerase in which the hybridized probe having a fluorescence material and a quencher is fragmented thereby resulting in interruption of quenching. In other words, the

method in Livak et al. is different from the present method in requiring the 5' nuclease PCR assay.

Further, in the method in Livak et al., quenching is interrupted whenever the probe hybridizes with the target nucleic acid. Specifically, quenching is interrupted by partial hybridization. This means that a single base mismatch cannot easily be detected by the method in Livak et al.

Therefore, in view of the above, it is respectfully submitted that one skilled in the art could make and use the present invention without undue experimentation.

Thus, the 112, first paragraph, enablement rejection is untenable and should be withdrawn.

CONCLUSION

In view of the foregoing amendments and remarks, it is respectfully submitted that the present application is in condition for allowance and early notice to that effect is hereby requested.

If the Examiner has any comments or proposals for expediting prosecution, please contact the undersigned attorney at the telephone number below.

Respectfully submitted,

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ATTACHMENTS

1. Relevant pages of the Dictionary of Biochemistry and Molecular Biology.

DICTIONARY OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

Second Edition

J. STENESH
Professor of Chemistry
Western Michigan University

WILEY

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JOHN WILEY & SONS

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proportional to the frequency of the radiation.

quantum yield The number of molecules that react chemically in a photochemical reaction, divided by the number of photons absorbed; the number of moles that react chemically in a photochemical reaction, divided by the number of einsteins absorbed.

quark *See* elementary particles.

quartet A quadruple peak, such as a nuclear magnetic resonance peak that has split into four peaks.

quartile A set of quantiles; specifically, the quartiles Q_1, Q_2, and Q_3 are values at or below which lie, respectively, the lowest 25%, 50% and 75% of a set of data.

quartz A glassy silicon dioxide that is used for the production of cuvettes that are utilized in absorbance measurements of ultraviolet light.

quasar Quasi-stellar radio source; a compact radio source with a star-like optical object; a "radio star."

quat Quaternary ammonium compound; one of a group of cationic detergents used as antiseptics and disinfectants. The compound cetyltrimethylammonium bromide (CTAB, cetavlon) is an example.

quaternary ammonium compound *See* quat.

quaternary nitrogen A positively charged nitrogen atom that is linked to other atoms or groups by means of four covalent bonds.

quaternary structure The structure of a protein that results from the interaction between individual polypeptide chains to yield larger aggregates; the arrangement in space of the subunits of a protein and the intersubunit contacts and interactions without regard to the internal structure of the subunits.

que Queuine.

queen substance Originally a term for the entire mandibular gland secretion of the queen bee which contains about 30 different substances; now a trivial name for the compound 9-oxo-*trans*-2-decenoic acid which serves as a pheromone for maintaining the division of labor in the beehive.

Quellung reaction The precipitin reaction that occurs between polysaccharides of bacterial capsules and antibodies to these polysaccharides; it results in an apparent swelling of the capsule.

quench correction curve A plot of counting efficiency versus the ratio of counts in two channels; used to correct the observed counts in liquid scintillation for quenching.

quenching 1. The process whereby secondary and subsequent ionizations in an ionization detector are stopped so that the detector becomes again sensitive to new, incoming ionizing radiation. 2. A decrease in the counting efficiency in liquid scintillation. 3. The decrease in fluorescence that results from an absorption of some or all of the emission energy. *See also* fluorescence quenching.

quetelet index A measure of obesity defined as the weight in kilograms divided by the square of the height in meters.

queuine A modified guanine found in tRNA. It differs from other modified (minor, rare) bases in that it is synthesized first as a base and then incorporated into mature tRNA by an enzyme-catalyzed exchange reaction in which guanine is removed from the tRNA and replaced by queuine. *Abbr* Q.

queuosine The ribonucleoside of queuine. *Abbr* Q; Quo.

quick-stop mutant A mutant of *E. coli* that immediately stops replication when the temperature is raised to 42°C.

quinacrine An acridine dye derivative that is a fluorochrome and that is used in the treatment of malaria and cancer.

quinary structure The group of macromolecular interactions between proteins that are transient in vivo.

quinine An alkaloid drug used in the treatment of malaria; a cinchona alkaloid.

quinoline alkaloids A group of alkaloids that contain the quinoline structure. They include the cinchona alkaloids, derived from the bark of tropical trees, especially *Cinchona succiruba*. The main alkaloid of the bark of *Cinchona* is quinine, a drug used for the treatment of malaria.

quinolizidine alkaloids *See* alkaloids.

quinone *p*-Dioxybenzene or a derivative of *p*-dioxybenzene. A particular quinone (coenzyme Q) serves as an electron carrier in the electron transport system.

quinone cycle A postulated mechanism for proton translocation according to the chemiosmotic coupling hypothesis; involves respiratory complex III and the participation of a semiquinone radical. A cyclic set of reactions in which coenzyme Q undergoes a two-stage reduction with the semiquinone as a stable intermediate.

quinoprotein One of a group of dehydrogenases that have the complex organic compound pyrrolo quinoline quinone (PQQ; methoxatin; 4,5-dihydro-4,5-dioxo-1*H*-pyrrolo-[2,3*f*]chinoline-2,7,9-tricarboxylic acid) as a prosthetic group. The latter serves as a coenzyme for the dehydrogenases that occur in methylotrophic and other types of bacteria.

quo Queuosine.

Q value The total energy per atom that is released in a nuclear reaction in which a nuclide is transformed into another, and ground-state, nuclide.